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EFFICACY OF SYRIAN PROPOLIS AGAINST SEVERAL BACTERIAL STRAINS

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ABSTRACT: Propolis is a natural material that can be found in bee honey. It has many medical uses due to its antibacterial activity. This study aimed to estimate the antibacterial efficacy of three propolis extracts using three different solvents (Ethanol, Methanol, and Ethyl Acetate). We experienced the three extracts on four different strains of bacteria (*Klebsiella pneumonia*, *E. coli*, *Pseudomonas aruginosa* & *Staphylococcus aureus*). The results indicated that all the studied propolis extractions do not have any efficacy on *Klebsiella* neither on *E. coli*. The methanolic extract has the greatest effect on *Staphylococcus aureus* as well as on *Pseudomonas aruginosa* in contrast to ethanolic and ethyl acetate extracts, which has no efficacy on any of the studied bacterial strains.

INTRODUCTION: Propolis is a natural resin material that is rich in effective compounds like flavanones such as Naringenin, pinobanksin, pinostrobin, pinocembrin, and sakuranetin ¹. It is widely used for the prevention and treatment of many diseases ². The propolis extract is used as an antibacterial, disinfecting, and antiinflammatory in oral preparations such is toothpaste ³. Its antibacterial efficacy is due to the presence of the flavor galagin, especially on Encountered cocci and Gram-positive bacteria ⁴. Propolis also contains aromatic acids such as caffeic, cinnamic, comaric, and ferulic in addition to esters such as benzyl caffeate, benzyl coumarate, and caffeic acid phenethyl ester ⁵.

The recent *in-vitro* studies has confirmed the antibacterial, antifungal, anti-inflammatory, anti-viral and anti-cancer activity of propolis such as its effect on human lung cancer which is still being studied ³. Its special effect was observed on bacterial species, with significant effect on Gram positive bacteria including the human tubercle bacillus, but only limited activity against Gram negative bacilli ⁶ and the therapeutic effectiveness is due to a combination of several active substances and not to a single effect of one substance ⁷. In this study, we aimed to evaluate the antibacterial effect of three different extracts of propolis, which were introduced with three types of solvents (ethanolic extract, methanolic extract and the ethyl acetatic extract on four different bacterial strains: *E. coli*, *Klebsiella*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

MATERIALS AND METHOD:

Preparation of Extracts: The propolis sample was obtained from Tartous, Syria in September 2019.

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To prepare the ethanolic extract, the propolis was crushed into soft powder, and thereafter 5 g of the propolis powder was mixed with 20 ml of 95% of ethanol to obtain 25% (w/v) propolis extract. The same thing was done with the other two extracts, but the only difference was the solvents; for example, in the second extract, we mixed the propolis powder with 20 ml of methanol, and in the third extract, we mixed it with 20 ml of ethyl acetate. Extraction was carried out at room temperature in the dark for 7 days with periodical handshaking of the extractions. The extractions were centrifuged, and the supernatants were termed as an ethanolic extract of propolis (EEP), methanolic extract of propolis (MEP), and ethyl acetate extract of propolis (ACEP). The concentration of the three extracts was 25%. Then each sample was filtered by a 0.45-micrometer filter and collected into a sterile tube.

Preparation of Mueller-Hinton Medium: We suspended 38 grams in 100 ml distilled water, heated until boiling to dissolve the medium, and then we sterilized the media by autoclaving at 15 lbs pressure (121 °C) for 15 min. Suspensions of microorganisms containing 10^6 cells/ ml were inoculated onto plate surfaces with a sterile cotton swab. Test plates (diameter 10 cm) were prepared with 20 ml of Mueller-Hinton agar (himedia laboratories Pvt. Ltd), and holes of 6 mm in diameter were punched in the agar plates using cork borer⁸. Each hole was filled with 50 μ l of EEP. The diameters of the growth inhibition zones around the holes were measured after incubation for 24 h at 37 °C.

RESULTS: When the inhibition zone of propolis extract against both bacterial groups was greater than 6 mm is considered active.

TABLE 1: DIAMETERS OF INHIBITION ZONE OF PROPOLIS EXTRACTS ON BACTERIAL STRAINS

	EEP	MEP	ACEP
<i>Escherichia coli</i>	----	11 mm	----
<i>Pseudomonas aeruginosa</i>	----	12mm	----
<i>Staphylococcus aureus</i>	----	15mm	----
<i>Klebsiella</i>	----	----	----

----No inhibition zone

DISCUSSION: Our results can be very useful in order to estimate the therapeutic value of Syrian propolis as an antibacterial material. The experiment indicated that *Klebsiella* and *E. coli* are

the least sensitive bacterial strains against propolis extractions. Although, we find high sensitivity of *Staphylococcus aureus* and *Pseudomonas aeruginosa* to MEP. According to the fact that *P. aeruginosa* has always been considered to be a difficult target for antimicrobial chemotherapy because of its ability to produce β -Lactamase and the low permeability of its outer membrane⁹, Propolis with its efficacy against *P. aeruginosa* – which our experiment has proved – can be an important material to fight *P. aeruginosa* resistance. Our results are in agreement with Grange et al., who found that dilution of 1: 20 in nutrient agar, the propolis extraction had no effect on *Klebsiella pneumonia*⁷. Even though that our results show no efficacy of ethanolic extracts on our studied bacterial strains. This difference between results may be explained by the influence of the environment on the chemical composition of propolis.

CONCLUSION: Propolis is one of the most important natural raw materials that we should benefit from its antibacterial effects on multi-drugs resistant bacteria. Going back to natural resources is important to find new safer and more effective compounds to beat super bug bacteria.

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